CLAIMS

We claim:

1. A composition comprising a cleavage structure, said cleavage structure comprising:

a) a target nucleic acid, said target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;

b) a first oligonucleotide complementary to said fourth region of said target nucleic acid;

c) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said target nucleic acid; and

d) a third oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid.

2. The cleavage structure of Claim 1, wherein said first region of said target nucleic acid has a length of eleven to fifty nucleotides.

3. The cleavage structure of Claim 1, wherein said second region of said target nucleic acid has a length of one to three nucleotides.

4. The cleavage structure of Claim 1, wherein said third region of said target nucleic acid has a length of six to nine nucleotides.

5. The cleavage structure of Claim 1, wherein said forth region of said target nucleic acid has a length of six to fifty nucleotides.

6. The cleavage structure of Claim 1, wherein one or more of said first, said second, said third and said fourth oligonucleotides contain a dideoxynucleotide on at the 3' terminus.

7. The cleavage structure of Claim 1, wherein said target nucleic acid is not completely complementary to at least one of said first, said second, said third and said fourth oligonucleotides.

8. The cleavage structure of Claim 7, wherein said target nucleic acid is not completely complementary to said second oligonucleotide.

9. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage means,

ii) a source of target nucleic acid, said target nucleic acid having a first regin,a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region;

iii) a first oligonucleotide complementary to said fourth region of said target nucleic acid;

iv) a second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

iv) a third oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid;

b) mixing said cleavage means, said target nucleic acid, said first oligonucleotide, said second oligonucleotide and said third oligonucleotide to create a reaction mixture under reaction conditions such that said first oligonucleotide is annealed to said fourth region of said target nucleic acid and wherein at least said 3' portion of said second oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said third oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products, each non-target cleavage product having a 3'-hydroxyl group; and

c) detecting said non-target cleavage products.

10. The method of Claim 9, wherein said cleavage means is a structure-specific nuclease.

11. The method of Claim 10, wherein said structure-specific nuclease is a thermostable structure-specific nuclease.

12. The method of Claim 11, wherein said nuclease is encoded by a DNA sequence selected from the group consisting of SEQ ID NOS:1-3, 9, 10, 12, 21, 30, and 31.

13. The method of Claim 9, wherein one or more of said first, second, and said third oligonucleotides contain a dideoxynucleotide at the 3' terminus.

14. The method of Claim 13, wherein following said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one labelled nucleoside triphosphate under conditions such that at least one labelled nucleotide is added to the 3'-hydroxyl group of said non-target cleavage products to generate labelled non-target cleavage products; and

b) detecting the presence of said labelled non-target cleavage products.

15. The method of Claim 14, wherein said template-independent polymerase is selected from the group consisting of terminal deoxynucleotidyl transferase and poly A polymerase.

16. The method of Claim 15, wherein said second oligonucleotide contains a 5' end label, said 5' end label being a different label than the label present upon said labelled nucleoside triphosphate.

17. The method of Claim 13, wherein following said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one nucleoside triphosphate under conditions such that at least one nucleotide is added to the 3'-hydroxyl group of

said non-target cleavage products to generate tailed non-target cleavage products; and

b) detecting the presence of said tailed non-target cleavage products.

18. The method of Claim 17, wherein said template-independent polymerase is selected from the group consisting of terminal deoxynucleotidyl transferase and poly A polymerase.

19. The method of Claim 18, wherein said second oligonucleotide contains a 5' end label.

20. A method of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising:

a) providing:

i) a cleavage means,

ii) a source of target nucleic acid, said target nucleic acid having a first region, a second region and a third region, wherein said first region is located adjacent to and downstream from said second region and wherein said second region is located adjacent to and downstream from said third region;

iii) a first oligonucleotide having a 5' and a 3' portion wherein said 5' portion of said first oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said first oligonucleotide contains a sequence complementary to said third region of said target nucleic acid;

iv) a second oligonucleotide having a length between eleven to fifteen nucleotides and further having a 5' and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a

sequence complementary to said second region of said target nucleic acid;

b) mixing said cleavage means, said target nucleic acid, said first oligonucleotide and said second oligonucleotide to create a reaction mixture under reaction conditions such that at least said 3' portion of said first oligonucleotide is annealed to said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said target nucleic acid so as to create a cleavage structure and wherein cleavage of said cleavage structure occurs to generate non-target cleavage products, each non-target cleavage product having a 3'-hydroxyl group; and

c) detecting said non-target cleavage products.

21. The method of Claim 20, wherein said cleavage means is a structure-specific nuclease.

22. The method of Claim 20, wherein said second region of said target nucleic acid has a length between one to 5 nucleotides.

23. The method of Claim 20, wherein one or more of said first and said second oligonucleotides contain a dideoxynucleotide at the 3' terminus.

24. The method of Claim 20, wherein said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one labelled nucleoside triphosphate under conditions such that at least one labelled nucleotide is added to the 3'-hydroxyl group of said non-target cleavage products to generate labelled non-target cleavage products; and

b) detecting the presence of said labelled non-target cleavage products.

25. The method of Claim 20, wherein said detecting said non-target cleavage products comprises:

a) incubating said non-target cleavage products with a template-independent polymerase and at least one nucleoside triphosphate under conditions such that at least one nucleotide is added to the 3'-hydroxyl group of said non-target cleavage products to generate tailed non-target cleavage products; and

b) detecting the presence of said tailed non-target cleavage products.